Study of Tyrosine Kinase Receptor Tie2 in Covid19 Associated Coagulopathy Patients

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Abstract
Background: Endothelial cells have an abundance of the receptor Tyrosine Kinase Tie2. Proteolytic cleavage and ectodomain release generate soluble Tie2 in Covid19 patients. **Aim:** to study role of Tie2 expression in coagulopathy associated COVID19 patients with and to evaluate the impact of this on disease outcome. **Subjects and methods:** our study was conducted at Clinical Pathology Department and quarantine unit of Internal Medicine Department, it was a case control study on 50 patients with Covid19 having coagulopathy compared to 50 alike healthy controls admitted to Zagazig University Hospitals. Patient history, physical examination, routine laboratory tests, and flowcytometric evaluation of Tie2 expression were all performed to each participant. **Results:** there was statistically significant relation between the studied groups regarding platelet count (lower in case group), while all of prothrombin time, INR, partial thromboplastin time, serum ferritin, CRP, LDH, procalcitonin, D dimer, ESR and IL-6 were statistically significant higher in the case group. There was statistically significant relation between the studied groups regarding Tie2 expression (higher in case group). The primary outcome was evaluated at the end of the hospital stay and the patient group was sub-grouped into survivors and non-survivors. Out of the 50 study patients, 21(42%) were survivors and 29 (58%) were non-survivors. There was a statistically significant relation between outcome of case group and Tie2 expression (significantly higher in non-survivors patients). **Conclusion:** Tie2 may be an early and useful predictor of COVID19 clinical course, and it could be a relevant part of disease pathogenesis.

Keywords: Tyrosine Kinase Receptor, Tie2, Covid19, Coagulopathy.

Introduction

SARS-coronavirus 2 is a novel RNA virus that causes a severe respiratory illness known as coronavirus disease 2019 (COVID-19). The number of people infected with COVID-19 has skyrocketed since the first SARS-CoV-2 cases were reported in December 2019 in China. **(1)**.

Coronaviruses are positive single-stranded RNA viruses that are enclosed. Pneumonia was the first clinical indication of the SARS-CoV-2-related disease Covid-19, allowing case discovery. **(2)**.

COVID-19, a severe strain of SARS-CoV-2, was spreading rapidly over the globe (i.e., delta, lambda, mu, and omicron). Eighty percent of people infected with SARS-CoV-2 had mild to moderate symptoms (stage I). The remaining 20% may develop into terminal stages of illness within a week (stage II). After that, some people in stage II (about 5% of the population) may progress to stage III, which can lead to intubation or death. **(3)**.

Covid-19 patient is prone to coagulopathy and thrombotic complications. Compared to bacterial sepsis-induced coagulopathy (SIC) and disseminated intravascular coagulation (DIC), the symptoms of Covid-19-associated coagulopathy (CAC) are unique. D-dimer levels rise with CAC, but prothrombin time and platelet count show minimal abnormalities. Compared to SIC/DIC, CAC has a higher incidence of both

arterial and venous thromboembolisms. There is evidence that D-dimer can serve as a useful biomarker for assessing disease severity and predicting negative outcomes. **(4)**.

Endothelial cells have an abundance of the receptor Tyrosine Kinase Tie2. It promotes angiogenesis and is expressed by a specific macrophage subset. **(5)**.

Platelets and periendothelial cells release a protein called Angiopiotin-1 (Angpt-1) that stimulates Tie2. Angpt-1/Tie2 functions in endothelial cell proliferation, migration, and stability. **(6)**.

Endothelial alterations in septic DIC promote microvascular thrombus development by disrupting Tie2 signalling via Angiopiotin-1. Soluble Tie2 is produced in Covid19 patients when the cell surface full-length receptor is proteolytically cleaved and the ectodomain is released. **(7)**.

The study objective was to study role of Tie2 expression in COVID19 associated coagulopathy patients in a trial to brighten the aftereffect of this expression on disease outcome.

PATIENTS & METHODS

It is a case control study that was conducted at Clinical Pathology and quarantine unit of Internal Medicine Departments, Faculty of Medicine, Zagazig University from March 2021 to August 2022 on 50 patients with coagulopathy associated Covid19 compared to 50 matched apparently healthy controls. After receiving written agreement from all participants, the study was approved by researchers at Zagazig University's Medical Faculty. The study was conducted in accordance with the Declaration of Helsinki, a worldwide code of conduct for scientific investigations involving human participants. **IRB Approval No. (6769)**

Sample size:

The sample was calculated to be 100 subjects (50 cases, 50 controls). Informed written consents were obtained from all of them to use their samples and clinical data in this study. Patients were divided into 2 groups:

- **a.** Patient group: comprises 50 patients with COVID19 associated coagulopathy, identified via real-time reverse transcription polymerase chain reaction for SARS-Cov-2 detection in nasopharyngeal and oropharyngeal swabs. In total, there were 22 women and 28 men. Their ages varied from 46 to 71years, with 61.1±15.54 being the mean ±SD.
- **b.** Control group: contains fifty adults who appear to be in good health. There were 28 men and 22 women total. . Their ages varied from 42 to 68 years, with 62.64±13.64 being the mean ±SD. When it came to age and sex, they were a good fit for the patients.

Inclusion criteria:

1) Consent to participate in the research.
2) D-dimer levels above normal suggesting coagulopathy.

Exclusion criteria:

1) Patient refusal to share in the study.
2) Normal D-dimer.

Samling:

Peripheral blood samples were collected from all participants; samples were collected at the time of presentation. Venous blood samples were aseptically withdrawn from each participant by venipuncture. One ml of the blood sample was delivered into a sterile vacutainer containing EDTA for CBC examination and flowcytometric analysis of Tie2; 3 mLs were delivered into a sterile plain vacutainer tube for serum separation and 1.8 ml was delivered into a sterile vacutainer tube containing trisodium citrate for coagulation testing and D-dimer estimation.

All subjects were subjected to:

1. **Full history taking** Age, sex, symptoms of fever, loss of smell
2. **Complete clinical** was done particularly for fever.
3. **Abdominal ultrasound**
4. **Routine investigation including**:
   a) CBC: using of an XN 2000 automatic cell counter (Sysmex, Japan).

b) Coagulation tests (PT, PTT and INR): operated on automated blood coagulation analyzer, model CS 2500 (Sysmex, Japan).
c) Liver and kidney function tests, total creatine kinase, lactate dehydrogenase, serum calcium, phosphorus and magnesium assay: were performed by spectrophotometry using a manufacturer-supplied kit on Roche Cobas 8000, c702 module (Roche diagnostics, Switzerland).
d) Serum Ferritin, IL6 and procalcitonin: were carried out using the manufacturer-supplied reagent and a Roche Cobas 8000 autoanalyzer, e602 molecule by electrochemiluminescence (Roche diagnostics, Switzerland).
e) C- reactive protein and D-dimer: were carried out by immunoturbidimetric methodology utilising manufacturer-supplied reagent on a Roche Cobas 6000 autoanalyzer (c501 molecule) (Roche diagnostics, Switzerland).
f) Blood culture: The BACT/ALERT 3D equipment is used to incubate the culture bottles. Antibiotic susceptibility testing and bacterial identification were performed using the VITEK2 compact equipment on blood culture positive samples (Biomerieux, France).

5**. Specific research test: Flowcytometric assessment of Tie2 expression:**

Tie2 expression analysis was assayed by Becton Dickinson (BD) FACScan device (Franklin lakes, New Jersy, USA). Blood sample was manipulated quickly within 24 hours after withdrawal. For each sample, two tubes were labeled; one for the Tie2 expression analysis (Anti-Mo CD 202b) supplied by (Invitrogen, Carlsbad, California, USA) and the other tube for the negative control. Fifty microliters of the sample were delivered in each tube. Five microliters of Anti-Mo CD 202b were added to respective tubes of sample and isotypic control. The tubes were vortexed and incubated in the dark at room temperature for 15 minutes. 500 microliters of BD FACS lysing solution (Franklin lakes, New Jersy, USA) were added to each tube. The tubes were vortexed and incubated for 10 minutes in the dark at room temperature. The tubes were centrifuged at 1500 rpm for 5 minutes and the supernatant was discarded. Two milliliters of BD FACS lysing solution (Franklin lakes, New Jersy, USA) as a washing solution were added to each tube and mixed thoroughly. The tubes were centrifuged at 1500 rpm for 5 minutes and the supernatant was discarded. Cells were suspended in 500 microliters of BD FACS lysing solution to be ready for acquiring data by FACScan device as Tie2 expression is evaluated on the mononuclear cells.

Statistical Analysis

Analysis was performed using version 26 of SPSS. The median and the interquartile range were used to characterize the data, as well as the minimum and maximum values. The data obtained showed statistical significance at the 0.05% level. The Chi-square statistic was used in this analysis. Chi-squared test for trends was performed for ordinal-binary data. The distributional assumptions for parametric tests were checked with the use of the Kolmogorov-Smirnov and Levene tests. Independent sample t test was used to compare quantitative data between two groups when it was normally distributed, and the Mann Whitney test was employed when it was not. ROC curve was used to determine beast cutoff of certain quantitative parameter in diagnosis of certain health problem. Linear stepwise regression analysis was performed to measure associated independent factors for dependent factor and to predict the value of a variable based on the value of another variable.

RESULTS

Demographic and clinical charachterization:

Patient group involved 50 patients with COVID19 associated coagulopathy, there were 22 women and 28 men. Their ages ranged from 46 to 71years, with mean ±SD of 61.1±15.54.Control group consisted of fifty subjects, they were 28 men and 22 women, their ages varied from 42 to 68 years, with mean ±SD of 62.64±13.64. There was statistically non-significant relation between both groups regarding sex and age. Among case group, 30 patients (60%) had fever, 29 (58%) manifested with loss of smell, 5 (10%) had positive blood culture and 29 (58%) needed ventilation aids.

Comparison study between case and control groups:

There was statistically significant relation between the case and control groups regarding hemoglobin (lower in case group) ($p<0.001$), platelet count (lower in case group) ($p=0.043$) and total leucocytic count (higher in case group) ($p<0.001$). There was statistically significant relation between the studied groups regarding prothrombin time, INR, partial thromboplastin time, and D-dimer (higher in case group) ($p= 0.011$, 0.007, 0.049, <0.001 respectively). There was highly statistically significant relation between the studied groups regarding all of serum ferritin, CRP, LDH, total ceatinin kinase, procalcitonin, ESR and IL-6 (higher in case group) ($p<0.001$) for each. By comparing the cases to the controls regarding Tie2 expression, we found statistically significant relation between them as it was higher in case group ($p<0.001$) [Table 1].

Disease outcome:

The primary outcome was survival or mortality at the end of the hospital stay. We divided the patients into two groups (survivors and non-survivors).Out of the 50 cases, 21 cases (42%) discharged out of the quarantine unit, while 29 cases (58%) died.

Correlation between Tie2 expression and the other study parameters:

On applying correlation analysis to correlate between Tie2 expression and the other research analytes, there was statistically significant positive correlation between Tie2 expression and each of TLC, ESR, CRP, ferritin, D dimer, total protein, LDH, IL-6, total creatin kinase and serum procalcitonin level. While there was statistically significant negative correlation between Tie2 expression and each of haemoglobin level, serum calcium, and serum albumin level. On the other hand, there was non-significant correlation between Tie2 expression and any of the other tested parameters [Table 2].

Linear regression analysis of factors significantly correlated with Tie2 expression:

On implementing linear stepwise regression analysis of factors significantly correlated with Tie2 expression among studied participants, ESR (unstandardized $\beta=0.686$), D dimer (unstandardized $\beta=2.351$), serum albumin (unstandardized $\beta=-8.354$) and IL-6 (unstandardized $\beta=0.423$) were significantly independently associated with its expression [Table 3].

Tie2 exprssion as a predictor of Covid19 Associated Coagulopathy:

When the performance of Tie2 expression in predicting coagulopathy in our COVID19 patients was tested by applying ROC curve analysis, concluded that, the best cutoff of Tie2 expression in prediction of occurrence of coagulopathy in COVID19 case was ≥25.5% with area under curve 0.987, sensitivity 96%, specificity 92%, positive predictive value 92.3%, negative predictive value 95.8% and overall diagnostic accuracy of 94% ($p<0.001$) [Figure 1]

Relation between the outcome of the studied patients and laboratory practices:

In a practice to ameliorate the relation between the laboratory tested parameters and the prognostic outcome of the patient group, there was statistically significant relation between outcome and all of D- dimer, ferritin, CRP, procalcitonin and IL-6 level (higher in non-survivors patients). While there was statistically non-significant relation between outcome of case group and all of ESR, LDH, partial thromboplastin time, prothrombin time, INR, hemoglobin, total leucocytic count, platelet count, total-Ck, serum calcium, phosphorus or magnesium or any of the parameters of liver or kidney function test. There was statistically significant relation between outcome of case group and Tie2 expression (significantly higher in non-survivors patients) and this significance was high ($p<0.001$) [Table 4].

Tie2 exprssion as a predictor of mortality in Covid19 Associated Coagulopathy patients:

On performing ROC curve analysis to evaluate Tie2 expression level as a predictor of mortality among COVID19 patients, the best cutoff of Tie2 expression in prediction of mortality among COVID19 patients with coagulopathy was ≥73.05 with area under curve 0.813, sensitivity 79.3%, specificity 76.2%, positive predictive value 82.1%, negative predictive value 72.7% and overall diagnostic accuracy 78% ($p<0.001$) [Figure 2].

Table (1): Comparison between the studied groups regarding hematological data D-dimer, acute phase reactants, LDH, total creatine kinase, ESR and Tie2 expression

	Case group (N=50)	Control group (N=50)	t	p
	Mean ± SD	Mean ± SD		
Hemoglobin (g/dl)	11.46 ± 2.59(13.8-17)	13.18 ± 1.89	-3.788	**<0.001****
PT (seconds)	13.91 ± 3.18(10-13)	12.67 ± 1.06	2.611	**0.011***
INR	1.15 ± 0.26	1.04 ± 0.08	2.777	**0.007***
	Median (IQR)	**Median (IQR)**	**Z**	**p**
TLC (×10^3/ul)	11.25 (9.18 – 14)	7.55 (5.3 – 10.23)	-4.641	**<0.001****
Platelet count (10^3 /ul)	186.5(125 – 267.25)	203(165.75 – 335.25)	-2.027	**0.043***
PTT (second)	35.8 (32.65 – 41.3)	34.45 (29.98 – 37.25)	-1.969	0.049*
D dimer(ug/ml)	4.75 (2.3 – 6.7)	0.24 (0.09 – 0.35)	-8.619	**<0.001****
Ferritin (ng/ml)	840 (261.83 – 1226.5)	112.5 (73.5 – 257.28)	-6.794	**<0.001****
CRP (mg/l)	79.54 (32.64 – 161.69)	2.47 (1.6 – 4.05)2	-8.618	**<0.001****
LDH (IU/L)	498.5 (334 – 929)	166.5 (145 – 194)	-7.645	**<0.001****
Total creatine kinase (U/L)	220.5 (144.5 – 370.25)	100.5 (64.5 – 188.75)	-5.244	**<0.001****
Procalcitonin (ng/ml)	1.04 (0.74 – 2.37)	0.18 (0.09 – 0.35)	-8.412	**<0.001****
ESR (mm/hr)	42.5 (32 – 53)	4.92 (3.1 – 6.11)	-8.62	**<0.001****
IL-6 (pg/ml)	25.85 (20.1 – 32.8)	3.75 (2.2 – 5.2)	-8.618	**<0.001****
Tie2 expression %	78.53% (65.73 – 90.14%)	10.5% (6.98 – 14.24%)	-8.401	**<0.001****

Z Mann Whitney test, t independent sample t test, *p<0.05 is statistically significant, **p≤0.001 is statistically highly significant

Table (2): Correlation between Tie2 and the studied parameters

	r	P
Age (year)	-0.039	0.701
Hemoglobin (g/dl)	-0.366	**<0.001****
TLC(×10^3/ul)	0.314	**0.001****
Platelet count(×10^3/ul)	-0179	0.074
PTT (seconds)	0.104	0.304
PT (seconds)	0.155	0.125
INR	0.165	0.101
Calcium (mg/dl)	-0.214	**0.033***
Phosphorus (mg/dl)	-0.021	0.839
Magnesium (mg/dl)	0.073	0.472
Total protein (g/dl)	0.21	**0.036***
Albumin (g/dl)	-0.631	**<0.001****
Total bilirubin (mg/dl)	0.087	0.389
Direct bilirubin (mg/dl)	0.115	0.255
ALT(U/L)	-0.012	0.984
AST(U/L)	0.084	0.405
Creatinine (mg/dl)	-0.047	0.643
BUN (mg/dl)	0.136	0.177
Ferritin (ng/ml)	0.674	**<0.001****
CRP (mg/l)	0.723	**<0.001****
Total CK (U/L)	0.417	**<0.001****
LDH (IU/L)	0.662	**<0.001****
Procalcitonin (ng/ml)	0.771	**<0.001****
D dimer (ug/ml)	0.827	**<0.001****
ESR (mm/hr)	0.81	**<0.001****
IL-6 (pg/ml)	0.763	**<0.001****

r Spearman rank correlation coefficient, *p<0.05 is statistically significant, **p≤0.001 is statistically highly significant

Table (3): Linear stepwise regression analysis of factors associated with Tie2 expression

	Unstandardized		Standardized Coefficients	t	p	95.0% Confidence Interval	
	Beta	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	43.584	10.969		3.973	0.000	21.807	65.360
ESR (mm/hr)	0.686	0.113	0.446	6.061	0.000	0.461	.911
D Dimer (ug/ml)	2.351	0.727	0.219	3.232	0.002	0.907	3.794
Albumin (g/dl)	-8.354	2.520	-0.191	-3.315	0.001	-13.358	-3.351
IL-6 (pg/ml)	0.423	0.182	0.175	2.324	0.022	0.062	0.784

*p<0.05 is statistically significant, **p≤0.001 is statistically highly significant

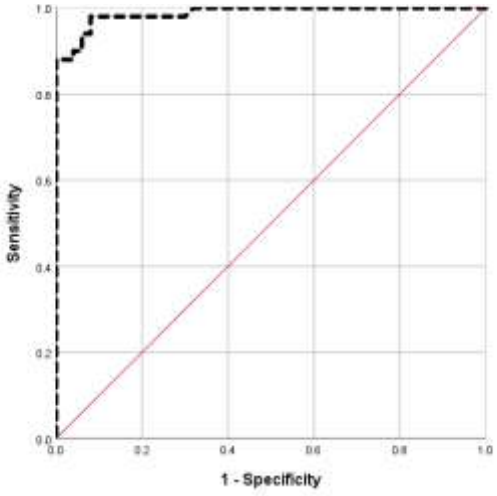

Figure (1): ROC curve showing performance of Tie2 in prediction of COVID19 associated coagulopathy.

Table (4): Relation between outcome of studied patients and tie2 expression along with other laboratory parameters

	Survivors N=21	**Non-survivors N=29**	**t**	**P**
	Mean ± SD	**Mean ± SD**		
Hemoglobin (g/dl)	12.01 ± 2.69	11.07 ± 2.27	1.267	0.211
PT (seconds)	14.45 ± 4.2	13.51 ± 2.17	1.031	0.308
INR	1.2 ± 0.34	1.11 ± 0.16	1.309	0.197
Total protein (g/dl)	7.94 ± 1.63	8.52 ± 2.58	-0.919	0.363
S albumin (g/dl)	3.1 ± 0.58	2.97 ± 0.66	0.768	0.446
Calcium (mg/dl)	9.78 ± 1.49	8.93 ± 1.51	1.967	0.055
Phosphorus (mg/dl)	3.33 ± 1.19	3.29 ± 1.1	0.123	0.903
Magnesium (mg/dl)	1.91 ± 0.41	2.13 ± 0.43	-1.864	0.068
	Median (IQR)	**Median (IQR)**	**Z**	**P**
Tie2 expression %	66.53 (50.24 – 75.65)	88.24 (75.96 – 90.55)	-3.745	<0.001**
Ferritin (ng/ml)	255 (147.5 – 772.95)	1000 (817.5 – 1764)	-4.511	<0.001**
CRP (mg/l)	50 (23.83 – 99.01)	100.9 (44.9 – 233.95)	-2.319	0.02*
LDH (IU/L)	450 (330 – 555)	500 (352 – 1000.54)	-0.953	0.34
Procalcitonin (ng/ml)	0.78 (0.57 – 1.1)	1.5 (0.9 – 3.15)	-3.018	0.003*
D dimer (ug/ml)	1.7 (1.2 – 2.4)	6.6 (5.1 – 9.1)	**-5.988**	**<0.001****
ESR (mm/hr)	37 (30 – 45)	46 (35 – 55)	-1.701	0.089
IL-6 (pg/ml)	20.9 (18.15 – 26.8)	29.6 (22.55 – 38.7)	-3.086	0.002*
TLC(×10^3/ul)	10.5 (6.7 – 14.1)	11.9 (9.7 – 13.8)	-0.954	0.34
Platelet count (×10^3/ul)	166 (78 – 239)	193(151 – 263)	-0.836	0.403
PTT (seconds)	33.3 (30 – 38.8)	34.9 (31.8 – 38.6)	-0.678	0.497
Total bilirubin(mg/dl)	0.52 (0.3 – 0.78)	0.51 (0.3 – 0.79)	-0.02	0.984
Direct bilirubin (mg/dl)	0.18 (0.1 – 0.27)	0.23 (0.13 – 0.38)	-1.151	0.25
ALT(U/L)	18.6 (12.5 – 33)	20 (15.6 – 27.8)	-0.138	0.891
AST(U/L)	25.7 (14.2 – 32.4)	28.5 (19.4 – 41.6)	-0.806	0.42
Creatinine (mg/dl)	0.77 (0.64 – 0.91)	0.84 (0.55 – 1.17)	-0.079	0.937
BUN (mg/dl)	24.5 (11.7 – 35.8)	26.8 (17.2 – 38.4)	-0.265	0.791
CK-total (U/L)	217 (180 – 431)	224 (145 – 299)	-0.708	0.479

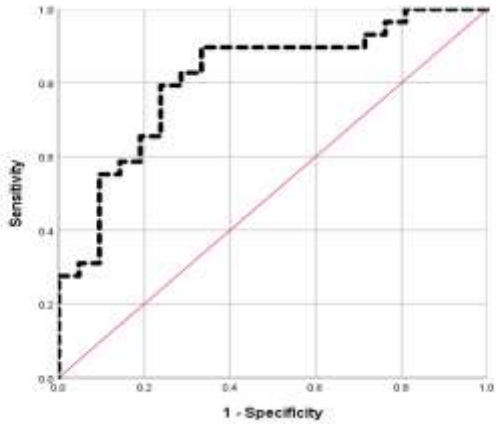

Figure (2): ROC curve showing performance of Tie2 in prediction of mortality among COVID19 associated coagulopathy patients

DISCUSSION

Although COVID-19 has unique characteristics, many patients with severe disease present with coagulation abnormalities that are similar to those seen in patients with other systemic coagulopathies associated with severe infections, such as disseminated intravascular coagulation (DIC) or thrombotic microangiopathy. Patients with COVID-19 who develop coagulopathy are more likely to die. **(4).**

Endothelial cells have an abundance of the receptor Tyrosine Kinase Tie2. It promotes angiogenesis and is expressed by a specific macrophage subset. **(8).**

In the current work, the results demonstrated that there was statistically non-significant relation between the case and the control group regarding gender and age. In agreement with our study, **Eslamijouybari et al. (9) and Hosseinpoor et al. (10)** found no statistically significant difference in regard to age or gender between cases and controls.

Among case group of this research, 60% had fever, 58% had loss of smell, 10% had positive blood culture and 58% needed ventilation aids**.** In agreement with our study, **Temiz et al. (11)** found that fever, exhaustion, and myalgia were the top three presenting symptoms, with cough coming in a distant fourth. Diarrhea, headache with dizziness and shortness of breath were the least common initial symptoms.

About haematological results in this work, there was statistically significant relation between the studied groups regarding haemoglobin ($p<0.001$), platelet count ($p=0.043$), both were lower in case group, and total leucocytic count that was higher in case group ($p<0.001$). In agreement with the study, **Pozdnyakova et al. (12)** demonstrated an increase in WBC count in COVID19 patients compared to controls.

In respect of coagulation tests in the ongoing study, there was statistically significant relation between the studied groups regarding prothrombin time ($p =0.011$), INR ($p=0.007$), partial thromboplastin time ($p=0.049$) and D dimer ($p<0.001$). In agreement with us, **Marcel et al. (13)** found that elevated D-dimer concentration and prolonged prothrombin time were exhibited as test findings in patients with COVID19 having coagulopathy. In disagreement with our study, **Chunjin et al. (14)** showed that PT was longer in the severe than the non-severe group, although the difference was not statistically significant.

In this work, there was statistically significant relation between the studied groups regarding serum ferritin, CRP, LDH, total creatin kinase, procalcitonin, ESR and IL-6. All of them were higher in case group with p value of <0.001 for each.

In agreement with this study, **Pozdnyakova et al. (12)** demonstrated elevated levels of serum ferritin and C-reactive protein in COVID-19 individuals. This may be because a fraction of COVID-19-positive individuals with more severe illness presentation exhibit a hyperinflammatory state. **(15). del Valle-Mendoza et al. (16)** showed that COVID 19 patients had a considerably higher mean IL-6 level compared to controls. In the work investigated by **Sarhat et al. (17),** elevated lactate dehydrogenase levels and cytokine-mediated tissue damage were described in patients with COVID19.

In this work, there was statistically significant relation between the two studied groups regarding Tie2 expression that was higher in case group with median expression of 78.53% in the patient group versus 10.5% in the control group. **Villa et al. (18)** revealed that endothelial inflammation is increased and vascular integrity is compromised when angiopoietin2 binds to its receptor (Tie2). Also, patients with ARDS and coagulopathy have been demonstrated to have a close relationship between the angiopoietin-2/1 ratio, circulating levels of angiopoietin2 and their prognosis.

When we checked out the patients to judge for their matter of course, 29 out of the 50 cases (58%) died while 21 cases (42%) discharged. These results were midway among different studies with different mortality reports**.(19-23)**

The difference in mortality rate could be attributed to the variable severity of COVID19 between the studied patients in the different studies with different age groups and comorbidities.

In this study, there was statistically significant positive correlation between Tie2 expression and each of TLC, ESR, CRP, ferritin, D dimer, total protein, LDH, IL-6, total creatin kinase and serum procalcitonin. While, there was statistically significant negative correlation between Tie2 expression and each of hemoglobin, serum calcium, and serum albumin level.

In agreement with the study, **Villa et al. (18)** found that inflammatory indicators, compromised vascular integrity, and increased endothelial inflammation are all linked Tie2 activation increased angiopoietin-2.

Schmaier et al. (21) reported that during systemic inflammation, increased expression of angiopoietin2 is linked to hypercoagulation and pathological thrombosis. Both soluble Tie2 and angiopoietin2 are up-regulated in severe sepsis, as they are in COVID-19. Taken together, their results expressed that the states of infectious disease characterised by severe inflammation had lower levels of Tie2 signalling. Also, they suggested that Tie2 activation cannot be reliably evaluated from measurements of soluble Tie2 or circulating angiopoietin-2. In COVID-19, they denoted that endothelial malfunction could be considered as a consequence of derangement of Tie2/angiopoietin-2 signalling and could be a cause of the subsequent procoagulant changes of the endothelium.

In this study, on performing linear stepwise regression analysis of factors significantly correlated with Tie2 expression among studied patients, ESR, D dimer and serum albumin and IL-6 were significantly independently associated with it**.**

When the performance of Tie2 expression in predicting coagulopathy in our COVID19 patients was tested by applying ROC curve analysis, concluded that, the best cutoff of Tie2 expression in prediction of occurrence of coagulopathy in COVID19 cases was ≥25.5% with area under curve 0.987, sensitivity 96%, specificity 92%, positive predictive value 92.3%, negative predictive value 95.8% and overall diagnostic accuracy of 94%.

We were interested to compare between the discharged patients and those who died in hospital as regards the research parameters.

We found that there was statistically significant relation between outcome and all of D- dimer, ferritin, CRP, procalcitonin and IL-6 level that all were lower in survivor patients. These findings came to be concordant with other researches.**(24-28)**

While there was statistically non-significant relation between outcome of case group and all of prothrombin time, INR, partial thromboplastin time, hemoglobin, total leucocytic count, platelet count, ESR total-Ck LDH, serum calcium, phosphorus, magnesium or any of the parameters of liver or kidney function test. Our results came harmonious with other researches that were attentive to weigh the effect of Covid19 on patient outcome **(29-31)**. Our results as regards this statistically non-significant relation came divergent with **Baroiu et al. (28), Zheng et al. (32)** and **Ayranci et al. (33)** who ascertained the existence of a statistically significantly difference in the tested parameters between the survivor and non- survivor groups. This divergence could be ascribed different sample size and discrepancy in disease severity.

In the present study, the median expression level of Tie2 was 66.53% in the survivors, while the median expression was 88.24% in the non-survivors with statistically significant relation between Tie2 expression level and disease outcome that was significantly lower in survivor patients ($p<0.001$).

On performing ROC curve for Tie2 expression level as a predictor of mortality among COVID19 patients, the best cutoff of Tie2 expression in prediction of mortality among COVID19 patients with coagulopathy was ≥73.05 with area under curve 0.813, sensitivity 79.3%, specificity 76.2%, positive predictive value 82.1%, negative predictive value 72.7% and overall diagnostic accuracy 78%**.**

Li et al. (7) have determined that the angiopoietin-Tie2 pathway is a prognostic factor and predictor of mortality in COVID 19 patients. In addition, **Trent et al. (34)** identified endothelial impairment related to Tie2 that contributed to lung injury and mortality due to dysregulated pulmonary function.

Conclusion

Coagulation dysfunction is closely related to the severity of patients with COVID19, in which low platelets, high D-dimer, long prothrombine time and partial Thromboplastine time upon admission may serve as risk indicators for increased aggression of the disease.

Tie2 may be an early and useful predictor of COVID19 clinical course, and it could be a relevant part of disease pathogenesis.

Limitations and Recommendations

Due to the present study's small sample size and inclusion of only patients from one institution, additional multicenter studies with a bigger sample size are required to validate the findings.

In the near future, researches need to be done to determine how Tie2 expression could be a relevant part of Covid19 Associated Coagulopathy pathogenesis.

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